A COMPARATIVE STUDY OF SOME STRAINS RECEIVED AS NOCARDIAE[1]

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Received for publication June 18, 1956

The assembly of a collection of strains of *Nocardia* and *Streptomyces* and of rapidly growing strains of *Mycobacterium*, begun in 1951, has been continued,[2] and a comparative study initiated to find stable characteristics for the identification of the genera and species represented. Results of the examination of the colonial morphology of 214 strains labeled as *Nocardia*, 219 as *Streptomyces*, and 243 as *Mycobacterium* are described here and evaluated for use in the separation of the genera.

Two aggregates of strains, approximately 70 per cent of which were received as nocardiae, were believed to typify two distinct species. A group of properties for the generic and specific recognition of each is presented, and the strains assigned to the two taxa are listed with their histories.

MATERIALS AND METHODS

The tests and observations described by Gordon and Smith (1955*a*) were used with the following additions and amendments:

Microscopic examination. For the examination of colonial morphology, made according to the procedure previously outlined, the importance of a fairly heavy inoculum should be stressed. One loopful of a broth culture, if turbid, was sufficient for each tube of agar. Some broth cultures, particularly of *Streptomyces* and *Nocardia*, required thorough shaking and the use of two to three loopfuls as inoculum. The resulting growth on the microscope slide contained at least one colony per field under the 10 × objective.

Decomposition of xanthine. The method and medium for establishing the decomposition of tyrosine were applied with the substitution of 4 g of xanthine for 5 g of L-tyrosine.

Hydrolysis of gelatin. Duplicate plates of the following medium were streaked once across with each culture: peptone, 5 g; beef extract, 3 g; agar, 15 g; gelatin, 4 g; distilled water, 1,000 ml; pH 7.0. The gelatin was soaked in approximately 40 ml of cold water before mixing with the melted agar. After 5 days' incubation at 28 C, one plate was covered with 8 to 10 ml of the following solution: $HgCl_2$, 15 g; concentrated HCl, 20 ml; distilled water, 100 ml (Frazier, 1926). Hydrolysis of the gelatin was measured by a clear zone underneath and around the growth; unchanged gelatin formed a white, opaque precipitate. The second plate was incubated for 10 days before testing.

Hydrolysis of starch. The medium for determining hydrolysis of gelatin was used with the substitution of 10 g of potato starch for gelatin. The starch was also suspended in 40 ml of cold water before its addition to the melted agar. After autoclaving, the agar was carefully mixed and poured into plates. Duplicate plates were streaked and stored at 28 C. After 5 days' incubation, one plate was flooded with 8 to 10 ml of 95 per cent alcohol; the second plate, after 10 days. A clear zone surrounding the growth, in contrast to the opaque, unchanged starch, indicated the extent of hydrolysis (Kellerman and McBeth, 1912).

Reduction of nitrate to nitrite. Cultures were prepared in the following medium: peptone, 5 g; beef extract, 3 g; KNO_3, 1 g; distilled water, 1,000 ml; pH 7.0. After 5, 10, and 14 days' incubation at 28 C, 1 ml of the broth culture was withdrawn aseptically and mixed with 3 drops of each of the following solutions: (1) Sulfanilic acid, 8 g; 5 N acetic acid (1 part of glacial acetic acid to 2.5 parts of water), 1,000 ml. (2) Dimethyl-α-naphthylamine, 6 ml; 5 N acetic acid, 1,000 ml (Conn, 1951). The presence of nitrite was indicated by a red color. In the absence of nitrite after 14 days' storage, 4 to 5 mg of zinc dust was added to the tube previously tested for nitrite. The zinc reduced the nitrate, if present, and produced a red color. Nitrate had to be demonstrated in the broth before a culture giving a negative

[1] This investigation was supported in part by research grant No. E 157-E 157(C4) from the National Institutes of Health, U. S. Public Health Service.

[2] The kind generosity of the many investigators who contributed their cultures for this project is gratefully acknowledged.

reaction for nitrite was reported as unable to reduce nitrate to nitrite.

Soil extract agar. The amount of air dried soil (1,000 g) was inadvertently omitted from the previous description. If the soil was rich in organic matter, only 500 g was autoclaved with 2,400 ml of tap water.

Survival of 60 C. The cultures were inoculated on slants of yeast dextrose agar, quickly heated to 60 C in a water bath, then transferred to a water bath at the same temperature inside a constant temperature incubator. After 4 hr they were quickly cooled, incubated at 28 C for 2 weeks, and examined for growth.

Temperatures of growth. Subcultures on yeast dextrose or Bennett's agar were made and immediately placed in a water bath at the desired temperature. After the cultures had reached the proper temperature, they were transferred to another water bath inside a constant temperature incubator. The water level and the temperature of the bath were carefully maintained. Growth was recorded after 5 to 7 days at temperatures of 35 C or above and after 3 weeks at 10 C.

Utilization of organic acids as carbon sources. Modifications of Koser's citrate agar (1924) were made by combining 2 g of Na acetate, Na benzoate, Na citrate, Na lactate, Ca malate, Na propionate, Na pyruvate, Na succinate, or Na tartrate with NaCl, 1 g; $MgSO_4$, 0.2 g; $(NH_4)_2HPO_4$, 1 g; KH_2PO_4, 0.5 g; agar, 15 g; distilled water, 1,000 ml; pH 6.8. After the adjustment of the pH, 20 ml of a 0.04 per cent solution of phenol red (indicator solution) was added. Use of an organic acid as a source of carbon by a culture was demonstrated by the alkaline color of the indicator after 4 weeks' incubation at 28 C.

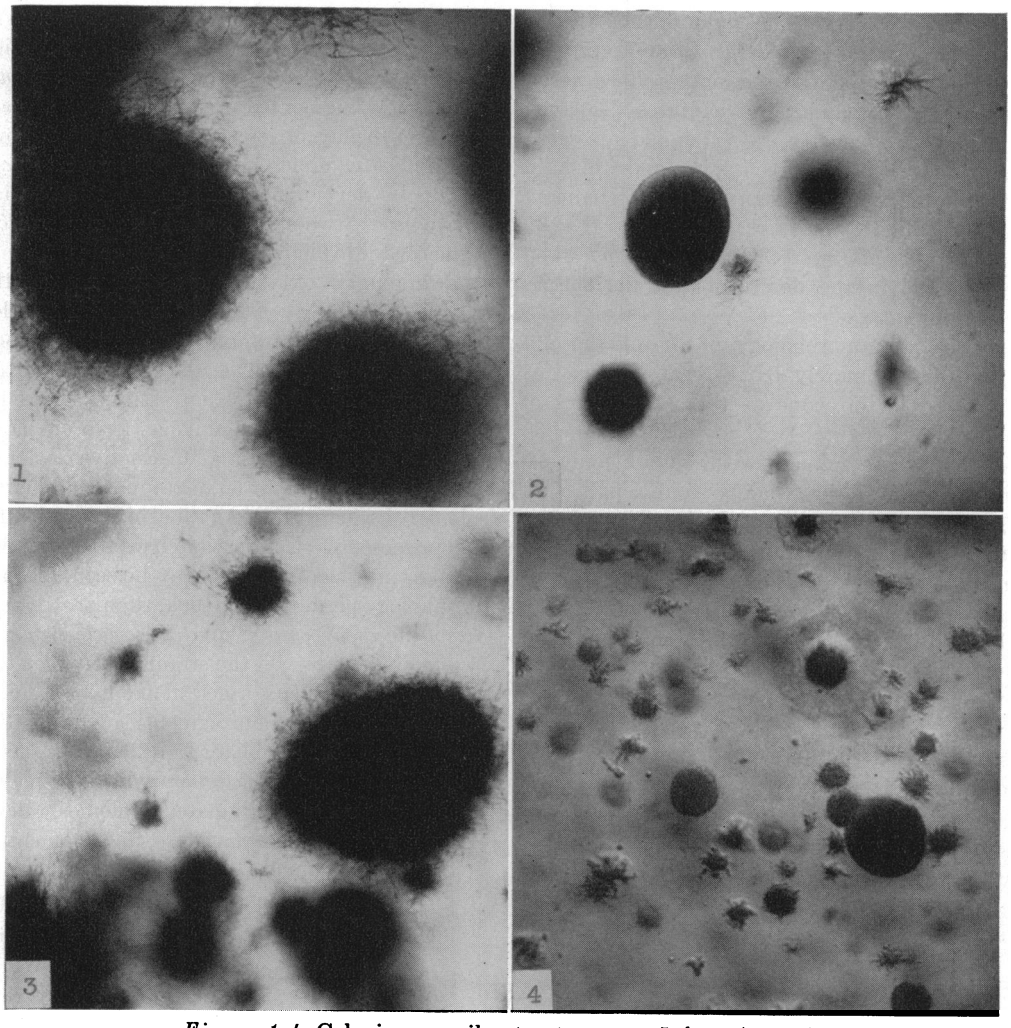
RESULTS

Colonial Morphology

Under the conditions of this examination, the 219 strains received as *Streptomyces* formed filamentous colonies (figure 1). When the agar was inoculated heavily enough to contain at least one colony per field (10 × objective), the rhizoid vegetative hyphae of each colony usually projected extensively into the agar and frequently interlaced with the hyphae of the surrounding colonies. Although very fine and slender, the hyphae appeared firm and resilient and rarely fragmented into rod and coccoid forms. Colonies with smooth margins were not observed. Approximately 92 per cent of the 219 strains of *Streptomyces* exhibited aerial hyphae, which seemed wider and darker than the vegetative hyphae in the unstained preparations. During the early formation of the aerial hyphae, their points of departure from the vegetative hyphae could be traced, and their free ends could be seen to move at any disturbance. Upon continued incubation, the aerial hyphae of 182 of the 219 strains segmented into chains of even, beadlike spores; 19 of the strains produced aerial hyphae but did not sporulate. The remaining 8 per cent (18 cultures) of the 219 strains did not reveal aerial hyphae, although the majority were known to be descendants of strains that had developed aerial hyphae and spores.

The appearance of the vegetative hyphae of the colonies grown on Bennett's and soil extract agars was the same. When Czapek's glucose asparagine, glycerol, and yeast dextrose agars were used, no differences in the vegetative hyphae were observed. Formation of aerial hyphae and sporulation, in some cases, were more affected by the medium. Some strains produced aerial hyphae and spores on Bennett's agar, but only rudimentary aerial hyphae on soil extract agar; other strains sporulated on soil extract agar, but not on Bennett's agar; and others formed spores on yeast dextrose agar, but not on soil extract or Bennett's agars.

Of the 243 strains received as *Mycobacterium*, approximately 89 per cent displayed dense colonies with smooth margins, dense colonies with a halo or outcroppings of filaments, and loosely filamentous colonies (figure 2). Figure 4 could also be an illustration of a culture of *Mycobacterium fortuitum*, *Mycobacterium phlei*, or *Mycobacterium smegmatis* growing in a smoother stage than culture No. 457 used in figure 2. The hyphae varied from short, rudimentary, and infrequently branching to extremely rhizoid with many very short branches on the longer hyphae. The rhizoid hyphae had a fragile, loosely connected, blurred appearance and frequently fragmented into rod and coccoid forms. Six per cent of the 243 cultures exhibited only dense colonies with smooth edges, while the remaining 5 per cent presented filamentous colonies and colonies with a halo or outcroppings of filaments. These colonial variations were not limited to one species. Three cultures of *M. phlei*, for example, displayed only dense colonies with smooth edges; two cultures produced only filamentous colonies; and the re-



Figures 1–4. Colonies on soil extract agar at 5 days (× 143)

Figure 1. Vegetative hyphae of *Streptomyces* sp. strain 3595.

Figure 2. Dense smooth colony and small filamentous colonies of *Mycobacterium fortuitum* strain 457.

Figure 3. Vegetative hyphae of *Nocardia asteroides* strain 436.

Figure 4. Dense, smooth colonies, colonies surrounded by fragmented filaments, and small filamentous colonies of *Mycobacterium rhodochrous* strain 372.

The authors are indebted to Mr. D. H. Braendle, Institute of Microbiology, Rutgers University, for the above microphotographs.

maining 28 of the 33 strains assigned to the species formed both.

Aerial hyphae similar to those of cultures of *Streptomyces* were not observed in colonies of mycobacteria on Bennett's agar, soil extract agar, or any of the other media used. Some colonies of nine cultures (3.7 per cent), however, did exhibit what appeared to be tufts of coalescing vegetative hyphae. One of the nine was a culture of *Mycobacterium phlei;* five were *Mycobacterium smegmatis;* two were *Mycobacterium fortuitum;* and one was not specifically identified. The presence of these tufts, which might at first glance be mistaken for aerial hyphae, was accompanied by dense colonies with smooth margins, filamentous colonies, and fragmenting hyphae. The over-all picture, therefore, was that of typical cultures of mycobacteria.

Under the conditions of these observations, 168 of the 214 strains received as *Nocardia* formed

filamentous colonies that could not be distinguished from those of *Streptomyces* (figure 3). Twenty-one of the 168 did not produce aerial hyphae. The remaining 147 cultures displayed aerial hyphae, which were wider and appeared darker than the vegetative hyphae and which, in the case of 49 cultures, segmented into chains of even, beadlike spores. Seventeen of the 49 sporulating strains were *Nocardia asteroides* and are included in the description of the species given below.

The colonial morphology of 38 of the 214 strains (approximately 18 per cent) received as *Nocardia* was the same as that of cultures of mycobacteria (figure 4). Thirty-four of the 38 developed dense colonies with smooth margins, dense colonies with a halo or outcroppings of filaments, and filamentous colonies. The colonies of the remaining four cultures were filamentous or dense with filamentous edges. After several days' incubation, the hyphae usually fragmented into rod or coccoid forms. Some of the filamentous colonies of three of the 38 cultures produced tufts of vegetative hyphae similar to those of the nine strains of mycobacteria. The three cultures otherwise resembled the ones illustrated in figures 2 and 4. Among these 38 strains were those received as *Nocardia corallina*, *Nocardia erythropolis*, *Nocardia globerula*, and *Nocardia opaca*. Two of these species, *N. erythropolis* and *N. opaca*, were studied and assigned to the α *Proactinomyces* by Umbreit (1939).

Although the vegetative hyphae of the eight remaining strains received as *Nocardia* were like the extensive hyphae typical of strains of *Streptomyces*, they fragmented in a way usually characteristic of the mycobacteria. Six of the eight, however, indicated a closer relationship to the *Streptomyces* by development of aerial hyphae, which in two cases (two strains of *Nocardia asteroides*) segmented into chains of beadlike spores.

A summary of these observations on the colonial morphology of the strains initially labeled *Streptomyces*, *Nocardia*, or *Mycobacterium* is presented in table 1. As expected in the case of related organisms, intermediate strains showed a progression, or formed a linkage, from one large group of strains with a distinctive colonial morphology to another large group with different colonial characteristics. Any line of separation for the purpose of classification had to be drawn through intermediates. As may be seen, colonial morphology permitted the separation of strains of *Streptomyces*, *Nocardia*, and *Mycobacterium* into two major groups: (1) the strains of *Streptomyces* and *Nocardia* which formed filamentous colonies with extensive, nonfragmenting vegetative hyphae and aerial hyphae, and (2) the strains of *Nocardia* and *Mycobacterium* which displayed dense, smoothly marginated colonies, dense colonies with filamentous edges, and filamentous colonies. The filaments of the colonies frequently fragmented into rod and coccoid forms. No aerial hyphae were produced. As to be expected, a

TABLE 1

Distribution of colonial characters of strains initially designated as Streptomyces, Nocardia, or Mycobacterium

Original Designation (No. of Strains)	Group 1			Group 2		
	Filamentous Colonies; Vegetative Hyphae Projecting Extensively, Often Interlacing, Firm, Rarely Fragmenting			Colonies with Halo or Outcroppings of Filaments and Filamentous Colonies; Filaments Rudimentary to Rhizoid, Fragile, Often Fragmenting; No Aerial Hyphae		Only Colonies with Smooth Margins
	Sporulating aerial hyphae	Nonsporulating aerial hyphae	No aerial hyphae	No colonies with smooth margins	Plus colonies with smooth margins	
	%	%	%	%	%	%
Streptomyces (219)	83	9	8	0	0	0
*Nocardia** (214)	24	47	10	2	16	0
Mycobacterium (243)	0	0	0	5	89	6

* One per cent intermediate between groups 1 and 2.

number of strains (approximately 13 per cent of the total 676) varied in some degree from the typical morphology of one group or the other. These included the strains of *Streptomyces* and *Nocardia* that did not present aerial hyphae, although their vegetative hyphae were extensive, nonfragmenting, and interlacing; the strains of *Nocardia* whose vegetative hyphae fragmented but gave rise to aerial hyphae; the strains of *Mycobacterium* and *Nocardia* that formed tufts of vegetative hyphae in addition to the colonial characteristics of the second group; the strains of *Mycobacterium* and *Nocardia* that did not reveal dense colonies with smooth edges, although their filaments were rudimentary to rhizoid, plentifully branched, and fragmenting; and the strains of *Mycobacterium* producing only dense colonies with smooth margins. With only two exceptions, these strains, whose colonial morphology did not strictly conform to that of one group or the other, could be assigned to one because they possessed one or more of the distinctive characteristics of that group. This separation of the 676 strains, therefore, seemed a reliable one.

During this study, 44 per cent of the strains of this collection have been specifically identified (Gordon and Smith, 1955*b*), and all the strains of each species placed in one morphological group or the other. Members of the same species did not show both types of colonial morphology. This division of the strains into two groups on the basis of their colonial appearance was, therefore, tentatively accepted as a means of generic demarcation. Its complete acceptance depends on confirmation by the specific identification of a much higher percentage of the strains.

Descriptions of species

Since the beginning of this collection and its examination, the majority of strains received as *Nocardia* congregated in two groups. The cultures of one group displayed extensive filamentous colonies with aerial hyphae; those of the second, the colonial morphology of the mycobacteria. A distinctive pattern of other characteristics was established for each taxon and tested by application to a number of strains believed sufficient to insure its reliability.

From time to time during the assembly of this collection, the same strain was obtained from different donors. Because of the possibility that dissimilar conditions of maintenance had caused some variation, the cultures were given different accession numbers and examined as separate strains.

Nocardia asteroides (Eppinger) Blanchard

According to the histories supplied by the donors, the 79 strains (table 2) typifying this species had been isolated from human and animal infections and from soil. Forty-three of the 79 strains were labeled *Nocardia asteroides* or its varieties; 21 as 17 other species; and 15 merely as *Nocardia*, *Actinomyces*, or *Streptomyces* spp.

Because of the meagerness of the original descriptions of some of the species listed in table 2 and human fallibility in maintaining stock cultures, it was very difficult to decide with any assurance on the authenticity of some of the named strains. The strains bearing the following names, however, were accepted as conforming to the original account of their respective species: *Actinomyces graminis* Topley and Wilson (1929), *Actinomyces sumatrae* Erikson (1935), *Nocardia asteroides* (Eppinger, 1891) Blanchard (1896), *Nocardia asteroides* var. *crateriformis* (Baldacci, 1938) Waksman and Henrici (1948), *Nocardia blackwellii* (Erikson, 1935) Waksman and Henrici (1948), *Nocardia caprae* (Silberschmidt, 1899) Waksman and Henrici (1948), *Nocardia farcinica* Trevisan (1889), *Nocardia minima* (Jensen, 1931) Waksman and Henrici (1948), *Nocardia polychromogenes* (Vallée, 1903) Waksman and Henrici (1948), *Nocardia sebivorans* Gorrill and Heptinstall (1954), and *Streptomyces rubescens* (Jarach, 1931) Waksman and Henrici (1948). Although the strains labeled *Nocardia asteroides* var. *gypsoides* (Baldacci, 1938) Waksman and Henrici (1948) and *Nocardia rhodnii* (Erikson, 1935) Waksman and Henrici (1948) were not in complete agreement with their first descriptions, there were reasonable grounds for the belief that they were descendants of the original cultures, and they were regarded as authentic.

The invalidity of the name *Nocardia cuniculi* Snijders (1924) borne by strain A 6864 was established by Erikson (1935). No record of the combination *Nocardia asteroides* var. *pseudocarneus* (strain 651) was found, and it was not believed to have been effectively published. Strain 651, however, corresponded to the first description of *Asteroides pseudocarneus* Puntoni and Leonardi (1935). Although effective publication of the names *Nocardia eppingerii* (strain 508) and

TABLE 2

Strains identified as Nocardia asteroides (Eppinger) Blanchard

Laboratory No.	Name When Received, and Source
W 3601	*Actinomyces graminis* Topley and Wilson; S. A. Waksman, Rutgers Univ.; G. J. Friou (patient Skoka)
427	*Actinomyces mexicanus* Boyd and Crutchfield; I. B. Christison, Duke Univ.; N. F. Conant (998)
W 3425	*Actinomyces sumatrae* Erikson; S. A. Waksman; ATCC (6864)
421	*Actinomyces* sp.; I. B. Christison (312); N. F. Conant (1017); K. E. Cox (chest fluid)
A 9504	*Nocardia asteroides* (Eppinger) Blanchard; ATCC; O. Felsenfeld (Madura foot)
A 9969, A 9970	*Nocardia asteroides;* ATCC; C. W. Emmons (9955, 9956, generalized actinomycosis)
W 3306, W 3308	*Nocardia asteroides;* S. A. Waksman
W 3599	*Nocardia asteroides;* S. A. Waksman; S. V. Keating
N 6761	*Nocardia asteroides;* NCTC; N. F. Conant
398 to 401, 403	*Nocardia asteroides;* C. W. Emmons, NIH (9977, 9966, 9955, 9974, 9976)
406	*Nocardia asteroides;* J. M. Coffey, N. Y. Dept. of Health (47295); J. B. Fischer; N. F. Conant
409, 411	*Nocardia asteroides;* J. M. Coffey (47459, sputum; 50250, abscess)
420	*Nocardia asteroides;* I. B. Christison (313); N. F. Conant (1004); P. Lacaz (5)
423	*Nocardia asteroides;* I. B. Christison (343); E. W. Netherton
429, 430	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2123, 2124); L. S. Suter
433	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2214); J. H. McCain (leg abscess)
434, 443, 535, 537 to 539	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2221, lung; 2338, foot sinus; 2388, sputum; 2399, chest sinus; 2412, lumbar abscess; 2413, sputum)
436 to 438	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2249, 2250, 2251); B. F. Fetter (130, 142, 8076, abscesses)
439	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2280); J. F. Mohn (brain abscess)
440	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2293); W. B. VandeGrift (abscess)
442	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2336); S. B. Salvin (2001)
504, 598	*Nocardia asteroides;* P. Thibault, Inst. Pasteur, Paris (probably Eppinger's; B541)
529	*Nocardia asteroides;* I. B. Christison; N. F. Conant (2279); F. Cohen (aspirated pus)
548, 612	*Nocardia asteroides;* E. N. Azarowicz, Univ. of Calif., Los Angeles (6, 13)
552	*Nocardia asteroides;* E. N. Azarowicz (50, dog) (Bohl *et al.*, 1953)
578, 579	*Nocardia asteroides;* L. Ajello, CDC, Chamblee, Ga. (74A, scalp abscess; 39, gluteal lesion)
618	*Nocardia asteroides* var. *cratiformis* (Baldacci) Waksman and Henrici; E. N. Azarowicz (35); CBS
W 3300	*Nocardia asteroides* var. *gypsoides* (Baldacci) Waksman and Henrici; S. A. Waksman; A. T. Henrici
651	*Nocardia asteroides* var. *pseudocarneus* (*Asteroides pseudocarneus* Puntoni and Leonardi); E. N. Azarowicz (112); A. G. Ochoa (389); N. F. Conant
A 6846	*Nocardia blackwellii* (Erikson) Waksman and Henrici; ATCC; NCTC (630); J. McFadyean
503	*Nocardia blackwellii;* J. B. Clark, Univ. of Okla.; ATCC (6846)
444	*Nocardia brasiliensis* (Lindenberg) Castellani and Chalmers; C. C. Campbell, Army Med. Services Grad. School (2493)
N 659	*Nocardia caprae* (Silberschmidt) Waksman and Henrici; NCTC (Lister)
A 6864	*Nocardia cuniculi* Snijders; ATCC; NCTC (1935); E. P. Snijders
508	*Nocardia eppingerii* (*Streptotrix* [*sic*] *eppingerii* Rossi Doria); P. Thibault (brain abscess)
W 3318	*Nocardia farcinica* Trevisan; S. A. Waksman (356)
W 3399	*Nocardia farcinica;* S. A. Waksman; W. A. Hagan; C. P. Fitch
611	*Nocardia farcinica;* E. N. Azarowicz (11); C. C. Campbell; ATCC (3318)

TABLE 2—*Continued*

Laboratory No.	Name When Received, and Source
N 6531	*Nocardia gardneri* (Waksman *et al.*) Waksman and Henrici; NCTC; A. D. Gardner
A 8674	*Nocardia minima* (Jensen) Waksman and Henrici; ATCC; NCTC (3489); H. L. Jensen
652	*Nocardia phenotolerans* (*Actinomyces phenotolerans* Werkman and Patrick); E. N. Azarowicz (110); A. G. Ochoa (1139); A. L. Carrión
W 3409	*Nocardia polychromogenes* (Vallée) Waksman and Henrici; S. A. Waksman; H. L. Jensen
W 3409A	*Nocardia polychromogenes;* S. A. Waksman; NCTC (3487)
653	*Nocardia rhodnii* (Erikson) Waksman and Henrici; E. N. Azarowicz (111); A. G. Ochoa (1028); D. Erikson
N 8595	*Nocardia sebivorans* Gorrill and Heptinstall; NCTC; R. H. Gorrill
A 7372	*Nocardia sylvodorifera* Castellani; ATCC; A. Castellani
W 3656	*Nocardia* sp.; S. A. Waksman; A. M. Kligman (patient Brant)
W 3661	*Nocardia* sp.; S. A. Waksman; C. A. Payne (51-0516, brain abscess)
W 3663	*Nocardia* sp.; S. A. Waksman; E. Haynes (abscess)
441	*Nocardia* sp.; I. B. Christison; N. F. Conant (2298); W. E. Bray (hand lesion)
551, 553, 649	*Nocardia* spp.; E. N. Azarowicz (49, pleural fluid; 52, Calif. soil; 171, Indian soil)
606, 608	*Nocardia* spp.; W. K. Harris, Univ. of Mass. (porcine lung, bovine mastitis)
648	*Nocardia* sp.; E. N. Azarowicz (126); C. C. Campbell
657	*Nocardia* sp.; C. C. Campbell (3392-54)
658	*Nocardia* sp.; C. C. Campbell (5311-54); B. Brinkerhoff (20922, pulmonary mycosis)
W 3655	*Streptomyces rubescens* (Jarach) Waksman and Henrici; S. A. Waksman; H. Umezawa (Z-5-2)
W 3045A, W 3045B	*Streptomyces* spp.; S. A. Waksman (soil)

Nocardia phenotolerans (strain 652) was not discovered, strain 508 conformed to the original account of *Streptotrix* (*sic*) *eppingerii* Rossi Doria (1891), and strain 652 with that of *Actinomyces phenotolerans* Werkman and Patrick (1932). Castellani (1955, *personal communication*) was unable to supply the reference to his description of *Nocardia sylvodorifera* Castellani (strain A 7372), and a search of the literature did not reveal it. Strain 427 received as *Actinomyces mexicanus* Boyd and Crutchfield (1921), strain 444, *Nocardia brasiliensis* (Lindenberg, 1909) Castellani and Chalmers (1913), and strain N 6531, *Nocardia gardneri* (Waksman *et al.*, 1942) Waksman and Henrici (1948) were regarded as misnamed because of their disagreement not only with the first accounts of their respective species but also with other strains generally approved as authentic.

According to the International Bacteriological Code of Nomenclature (Buchanan *et al.*, 1948), when two or more species are combined, the epithet of the first effectively named and described species must be applied to the resulting species. In this instance, as the specific epithet *farcinica* had priority over the others, the species should have been named *Nocardia farcinica*, and *Nocardia asteroides* reduced to synonymy. *Nocardia asteroides*, however, has become firmly established in human medicine and, as reviewed by Bohl *et al.* (1953), not uncommon in veterinary medicine. *Nocardia farcinica*, on the other hand, has nearly disappeared from scientific reports, as evidenced by its being listed only twice since 1937 (Luque, 1946; Mariat, 1954). Only three strains of *Nocardia farcinica*, two of which were originally identical, were donated to this collection, indicating that *Nocardia farcinica* has also nearly disappeared from the larger culture collections and the collections of investigators interested in this taxon. In contrast, 43 of the 79 strains representing this species were received as *Nocardia asteroides* or its varieties. There was also, as previously stated, a possibility that the interpretation of the original account of *Nocardia farcinica* was a mistaken one and that strains W 3318, W 3399, and 611 did not typify *Nocardia farcinica*. For these reasons, no effort was made to dislodge the specific name *Nocardia asteroides* from general acceptance.

Microscopic appearance. The cellular morphology of these 79 strains grown on glycerol agar

varied from coccoid forms to long, tangled, branching filaments. Nine cultures showed coccobacilli to short, branching filaments; four presented only rods of varying lengths; 44, rods and filaments; and 22, long and branching filaments. Forty-three strains exhibited some degree of acid fastness, ranging from an occasional acid fast cell to 10 to 80 per cent of the rods and filaments.

In an attempt to modify the examination for acid fastness, 1 per cent H_2SO_4 was substituted for the acid alcohol used in the Ziehl-Neelsen method. During the comparison of strains, more cultures of *Nocardia asteroides* did reveal acid fastness than when decolorized with acid alcohol, but some cultures of *Streptomyces* were also acid fast. For this reason, the modification was not adopted.

All strains formed filamentous colonies with aerial hyphae. Twelve displayed only rudimentary aerial hyphae, while those of the remaining strains varied from short, gnarled, and branching to long and straight with little to much branching. The aerial hyphae of a few cultures produced spirals; those of a few branched in whorls; and those of 21 strains[3] segmented into chains of beadlike spores. Under the conditions of the examination, fragmentation of the vegetative hyphae was observed in only four cultures.

Macroscopic appearance. One of the most mutable characteristics of the strains of this species was the appearance of their growth on agar slants. After 12 to 14 days' incubation at 28 C on yeast dextrose agar, the growth varied from scant, flat, and restricted to abundant, spreading, and finely wrinkled to deeply folded. Pigments ranged from cream colored to pale yellow to beige to orange to deep pink. Whitish aerial hyphae were abundant in some cultures, scant in some, and invisible to the unaided eye in others. Of the 79 cultures 22 developed an amber to light brown, soluble pigment.

It was very easy to understand how a culture of *Nocardia asteroides* that formed acid fast coccobacilli, rods, and short filaments and whose growth was heavy, finely to coarsely wrinkled, cream colored to orange, and without noticeable aerial hyphae, could be mistaken for a culture of *Mycobacterium*. On the other hand, a culture of *Nocardia asteroides* that produced nonacid fast, long, tangled filaments and a cream colored, pale yellow, or beige growth thickly covered with whitish aerial hyphae, could as easily be accepted as a culture of *Streptomyces*.

Results of the physiological tests applied to the 79 strains of *Nocardia asteroides* are presented in table 3. It was believed that the following characteristics were the most valuable in the identification of the species: development of filamentous colonies with aerial hyphae; failure to hydrolyze

TABLE 3

Physiological characteristics of 79 strains of Nocardia asteroides

Property	Positive Strains
	%
Decomposition of:	
Casein	0
Gelatin	34
Tyrosine	0
Xanthine	0
Hydrolysis of starch	54
Acid from:	
Arabinose	0
Erythritol	4
Galactose	24
Glucose	100
Glycerol	100
Inositol	2
Lactose	0
Maltose	5
Mannitol	0
Mannose	16
α-Methyl-D-glucoside	0
Raffinose	0
Rhamnose	35
Salicin	25
Sorbitol	0
Xylose	0
Nitrite from nitrate	86
Growth at:	
50 C	24
45 C	39
40 C	88
35 C	100
28 C	100
10 C	14
Utilization of:	
Acetate	100
Benzoate	0
Citrate	33
Lactate	31
Malate	100
Propionate	100
Pyruvate	100
Succinate	100

[3] Strains received as Actinomyces were not included in the previous discussion of colonial morphology.

casein and to dissolve the crystals of tyrosine and xanthine; acid production from glucose and glycerol; lack of acid formation from arabinose, lactose, mannitol, inositol, and xylose; and utilization of acetate, malate, propionate, pyruvate, and succinate.

Mycobacterium rhodochrous (Overbeck) nov. comb.

Several of the 56 strains representing this species (table 4) have survived in collections for a long time as cultures for exhibition, because of their hardiness and striking orange or red pigments.

After examination of the first descriptions, it was believed that the strains bearing the following 16 names were in agreement with the original cultures: *Bacillus rubricus* Hefferan (1904), *Micrococcus rhodochrous* Overbeck (1891), *Mycobacterium agreste* Gray and Thornton (1928), *Mycobacterium eos* Büttner (1926), *Mycobacterium luteum* Söhngen (1913), *Mycobacterium rubropertinctum* (Hefferan, 1904) Ford (1927), *Nocardia erythropolis* (Gray and Thornton, 1928) Waksman and Henrici (1948), *Nocardia globerula* (Gray, 1928) Waksman and Henrici (1948), *Nocardia opaca* (den Dooren de Jong, 1927) Waksman and Henrici (1948), *Nocardia rubra* (Krassilnikov, 1941) Waksman and Henrici (1948), *Proactinomyces erythropolis* (Gray and Thornton, 1928) Jensen (1932), *Proactinomyces globerulus* (Gray, 1928) Reed (1939), *Proactinomyces ruber* Krassilnikov (1941), *Rhodococcus rhodochrous* (Overbeck, 1891) Zopf (1891), *Serratia havaniensis* (Sternberg, 1892) Bergey *et al.* (1923), and *Serratia rosea* Bergey *et al.* (1923).

An error in the descriptions accompanying the first appearance of the names *Serratia corallina* Bergey *et al.* (1923) and *Nocardia corallina* (Bergey *et al.*, 1923) Waksman and Henrici (1948) raised a technical question concerning the authenticity of the strains bearing these names. Bergey *et al.* (1923) gave the name *Serratia corallina* to *Bacillus mycoides corallinus* Hefferan (1904), which was later changed to *Nocardia corallina* by Waksman and Henrici (1948). Because a trinomial has no standing in nomenclature (Buchanan *et al.*, 1948), Hefferan was not cited as an authority for *S. corallina*. Part of the species' descriptions given by Bergey *et al.* (1923) and Waksman and Henrici (1948), however, was taken from Hefferan's account of *B. mycoides*

TABLE 4

Strains identified as Mycobacterium rhodochrous (Overbeck) nov. comb.

Laboratory No.	Name When Received, and Source
369	*Bacillus rubricus* Hefferan; R. S. Breed, N. Y. Agr. Exp. Sta. (He—11); Král Collection
368	*Bacterium cycloclastes* Gray and Thornton; R. S. Breed (2572); NCTC
A 271	*Bacterium mycoides* Migula; E. M. Weber, Chas. Pfizer & Co.; ATCC; E. O. Jordan
W 21	*Micrococcus rhodochrous* Overbeck; S. A. Waksman, Rutgers Univ.; R. S. Breed
365 to 367	*Mycobacterium agreste* Gray and Thornton; R. S. Breed (2562 to 2564); NCTC
462	*Mycobacterium eos* Büttner; M. T. Clement, NRC, Ottawa (500); R. Y. Stanier; O. von Plotho
N 8154	*Mycobacterium lacticola* Lehmann and Neumann; NCTC (Pellegrino)
587	*Mycobacterium luteum* Söhngen; A. J. Kluyver; N. L. Söhngen
583	*Mycobacterium phlei* Lehmann and Neumann; A. J. Kluyver; N. L. Söhngen
590	*Mycobacterium phlei;* J. Glover, Univ. of Liverpool (Pellegrini); A. Lutz
388	*Mycobacterium rubropertinctum* (Hefferan) Ford; R. S. Breed (279); H. L. Jensen
N 8139	*Mycobacterium* sp.; NCTC (Grassberger)
452, 453	*Mycobacterium* spp.; D. W. Bruner, Cornell Univ. (127, 129); W. Willie (soil)
499	*Mycobacterium* sp.; M. Panisset, Univ. of Montreal (Pellegrini 5A)
515	*Mycobacterium* sp.; G. Pacheco, Inst. Oswaldo Cruz; H. de Souza (213, sputum)
A 4273	*Nocardia corallina* (Bergey *et al.*) Waksman and Henrici; ATCC; H. J. Conn; P. H. H. Gray (0-3)
W 3406	*Nocardia corallina;* S. A. Waksman; ATCC (999); R. S. Breed (KBMC); Král Collection
W 3408	*Nocardia corallina;* S. A. Waksman; ATCC (4273); H. J. Conn; P. H. H. Gray (0-3)
502	*Nocardia corallina;* J. B. Clark, Univ. of Okla.; ATCC (4273)
624	*Nocardia corallina;* E. N. Azarowicz, Univ. of Calif., Los Angeles (56); ATCC (999)
A 4277	*Nocardia erythropolis* (Gray and Thornton) Waksman and Henrici; ATCC; H. J. Conn; P. H. H. Gray
W 3407	*Nocardia erythropolis;* S. A. Waksman; ATCC (4277); H. J. Conn; P. H. H. Gray

TABLE 4—*Continued*

Laboratory No.	Name When Received, and Source
A 9356	*Nocardia globerula* (Gray) Waksman and Henrici; ATCC; P. H. H. Gray
417	*Nocardia globerula;* W. C. Haynes, U. S. Dept. Agr. (NRRL, B-1306); ATCC (9356)
544	*Nocardia globerula;* E. N. Azarowicz; W. C. Haynes
N 576	*Nocardia lutea* (Erikson) Waksman and Henrici; NCTC (Khartoum)
680	*Nocardia lutea;* E. N. Azarowicz (147); Inst. Pasteur, Paris
A 4276	*Nocardia opaca* (den Dooren de Jong) Waksman and Henrici; ATCC; H. J. Conn; P. H. H. Gray
660	*Nocardia opaca;* E. N. Azarowicz (115); D. M. Webley
N 6117	*Nocardia rhodnii* (Erikson) Waksman and Henrici; NCTC (R3)
W 3639	*Nocardia rubra* (Krassilnikov) Waksman and Henrici; S. A. Waksman; G. Giolitti
415	*Nocardia rubra;* W. C. Haynes (NRRL, B-685); G. A. Ledingham
546	*Nocardia rubra;* E. N. Azarowicz (2, Cassano Baldacci)
482	*Nocardia* sp.; S. McMillen, Army Med. Services Grad. School (3393-red, sputum)
567	*Nocardia* sp.; N. M. McClung, Univ. of Kansas (12, oil sand)
620, 621	*Nocardia* spp.; E. N. Azarowicz (40, soil; 41)
674	*Nocardia* sp.; E. N. Azarowicz (sputum after NaOH digestion)
492	*Proactinomyces erythropolis* (Gray and Thornton) Jensen; P. H. H. Gray, Macdonald College (53)
494 to 496	*Proactinomyces globerulus* (Gray) Reed; P. H. H. Gray (M47, R48, S49)
565	*Proactinomyces polychromogenes* (Vallée) Jensen; N. M. McClung (6); CBS
562	*Proactinomyces ruber* Krassilnikov; N. M. McClung (1); CBS
493	*Proactinomyces* sp.; P. H. H. Gray (44)
372	*Rhodococcus rhodochrous* (Overbeck) Zopf; R. S. Breed (KMRh); Král Collection; W. Migula
364	*Serratia corallina* Bergey *et al.;* R. S. Breed (1652); NCTC; Král Collection
370	*Serratia corallina;* R. S. Breed (KBMC); Král Collection
363	*Serratia havaniensis* (Sternberg) Bergey *et al.;* R. S. Breed (319)
371	*Serratia rosea* Bergey *et al.;* R. S. Breed (KBMR, Scholl I); Král Collection
381, 382, 386	Unidentified strains; R. S. Breed (155, 161, 154); R. E. Gordon (soil)

corallinus. By some mischance, a portion of Hefferan's (1902) account of *Bacillus rosaceus metalloides* was included in the description of *S. corallina*. As a result, the strains of *S. corallina* and *N. corallina* in this collection were in sharp disagreement with the alien portion of the species' description, although they conformed to Hefferan's delineation of *B. mycoides corallinus*.

The strains received as *Bacterium cycloclastes* Gray and Thornton (1928), *Bacterium mycoides* Migula (1900), *Mycobacterium lacticola* Lehmann and Neumann (1899), *Mycobacterium phlei* Lehmann and Neumann (1899), *Nocardia lutea* (Erikson, 1935) Waksman and Henrici (1948), *Nocardia rhodnii* (Erikson, 1935) Waksman and Henrici (1948), and *Proactinomyces polychromogenes* (Vallée, 1903) Jensen (1931) did not correspond to the original account of their respective species and were looked upon as misnamed.

The oldest specific epithet believed to be properly represented in this group of strains, therefore, was *rhodochrous* (Overbeck, 1891), and it was assigned to this species, in accordance with the rule of priority. Reasons for the proposed generic location of the species will be given after the listing of the characteristics of the taxon.

Microscopic appearance. When grown on glycerol agar, 41 of the 56 cultures revealed coccobacilli, coccobacilli to short rods, or coccobacilli to short rods to branching filaments. Rods and filaments were frequently vacuolar. The remaining 15 cultures were composed of rods and filaments of varying lengths without the coccoid forms. Eight of the 56 cultures on glycerol agar showed traces of acid fastness; the rest were nonacid fast. After growing in milk for 2 weeks at 28 C, more of the cultures were acid fast.

When first isolated, strains 381, 382, and 386 were as strongly acid fast as freshly isolated cultures of *Mycobacterium phlei* and *Mycobacterium smegmatis*. After one or two transfers, however, their acid fastness was abruptly lost.

With two exceptions, the colonies of the 56 strains were similar to those formed by strains of *Mycobacterium*. Fifty cultures displayed dense colonies with smooth margins, dense colonies with a halo or outcroppings of filaments, and filamentous colonies. The filaments of 45 of these 50 cultures fragmented. Some of the colonies of one of the fragmenting cultures also showed tufts or peaks of vegetative hyphae. Four cultures revealed dense colonies with outcroppings or a

TABLE 5

Physiological characteristics of 56 strains of Mycobacterium rhodochrous

Property	Positive Strains
	%
Decomposition of:	
Casein	0
Gelatin	3
Tyrosine	73
Xanthine	0
Hydrolysis of starch	97
Acid from:	
Arabinose	0
Dulcitol	0
Erythritol	0
Galactose	3
Glucose	97
Inositol	12
Lactose	0
Maltose	16
Mannitol	100
Melibiose	0
α-Methyl-D-glucoside	0
Raffinose	0
Rhamnose	3
Salicin	8
Sorbitol	100
Trehalose	93
Xylose	8
Nitrite from nitrate	83
Survival of 60 C, 4 hr	25
Growth at:	
50 C	5
45 C	51
40 C	73
35 C	91
28 C	100
10 C	100
Utilization of:	
Benzoate	80
Citrate	87
Lactate	100
Malate	98
Succinate	100
Tartrate	0

fringe of filaments and filamentous colonies. The filaments were like those of the mycobacteria and fragmented. The two remaining cultures were the only two that could not be assigned to one or the other of the groups described in the preceding section on colonial morphology. Although their extensive, interlacing hyphae resembled those of the *Streptomyces*, they fragmented into short rods and coccoid forms. No aerial hyphae were observed in any of the 56 cultures.

Macroscopic appearance. After 12 to 14 days' incubation at 28 C on yeast dextrose agar, the growth of these strains was moderate to abundant; spreading; moist, butyrous, and glistening to dull, waxy, finely or coarsely wrinkled; cream colored to orange to coral to deep pink. Four cultures formed amber to light brown, soluble pigment.

Table 5 contains the data obtained from the physiological examination of the 56 strains representing *Mycobacterium rhodochrous*. The most useful characteristics for the identification of this species were as follows: negative or very weak acid fastness of cells grown on glycerol agar; colonial morphology similar to that of the mycobacteria; failure to decompose casein and xanthine; acid production from mannitol and sorbitol; lack of acid formation from arabinose, dulcitol, galactose, lactose, melibiose, α-methyl-D-glucoside, raffinose, and rhamnose; growth at 10 C; and nonutilization of tartrate. Failure to decompose tyrosine and inability to use benzoate were not employed to exclude strains from this taxon, because the results of these tests did not correlate with each other or with the results of any other observation. When these reactions were positive, however, they assisted greatly in the identification of the strains.

DISCUSSION

In the generic assignment of the species bearing the specific epithet *rhodochrous*, the acid fastness of the 56 strains had to be taken into account. Although they were not so acid fast as strains of *Mycobacterium phlei* or *Mycobacterium smegmatis* (which in turn are not so acid fast as those of *Mycobacterium tuberculosis*) they could not be described as completely nonacid fast. Three of the strains were known to be acid fast when first isolated, and it was assumed that many of the strains received as *Mycobacterium* spp. were also acid fast upon isolation. The strains received as *Mycobacterium eos*, *Nocardia erythropolis*, and *Proactinomyces erythropolis* were originally described as weakly acid fast. The characteristic of acid fastness, reliable for *M. tuberculosis*, *M. phlei*, and others, was variable for this species.

As determined here, colonial morphology divided the strains received as *Streptomyces*, *Mycobacterium*, and *Nocardia* into only two groups, and the line of demarcation between the groups was tentatively considered valuable and reliable enough for the separation of genera. Allocation of strains with colonial morphology like that of the mycobacteria to the same genus

as *Nocardia asteroides*, for example, would have crossed this line of demarcation and could not be undertaken. Because the similarities between the strains of the species having the specific epithet *rhodochrous* and the mycobacteria were believed to outweigh the dissimilarity in degree of acid fastness, this species was provisionally located in the genus *Mycobacterium*. It was acknowledged, however, that classification is a matter of individual opinion, and only when many investigators form the same opinion from their own studies can a point of classification become accepted and useful. This tentative assignment was, therefore, offered as confirmation of the conclusions of Büttner (1926), Ford (1927), Gray and Thornton (1928), Söhngen (1913), and others who placed strains of this species in the genus *Mycobacterium*.

SUMMARY

Strains received as belonging to the three genera, *Streptomyces*, *Mycobacterium*, and *Nocardia*, were divided into two groups on the basis of their colonial morphology. As the separation provided appeared to be reliable, it was tentatively used for generic demarcation.

Characteristics for the identification of *Nocardia asteroides* (Eppinger) Blanchard and *Mycobacterium rhodochrous* (Overbeck) nov. comb. were described.

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